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Group II phospholipase A_2 induced by interleukin-1β in cultured rat gingival fibroblasts

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Previously, we reported the presence of group II-like phospholipase A_2 activity in the soluble fraction of rat gingiva. In the present study, we found that treatment of rat gingival cells with human recombinant interleukin-1β resulted in dose-dependent stimulation of intracellular and extracellular phospholipase A_2 activity. Antisera against group II phospholipase A_2 totally blocked the interleukin-1β-induced phospholipase A_2 activity, but antisera against group I phospholipase A_2 did not. Moreover, immunoblot analysis showed that the induced phospholipase A_2 was group II phospholipase A_2. These findings suggest that the induced enzyme belongs to the group II phospholipase A_2 family of proinflammatory enzymes.

Phospholipase A_2; Interleukin-1β; Rat gingival fibroblast; Periodontitis

1. INTRODUCTION

Phospholipase A_2 (PLA_2; EC 3.1.1.4) catalyzes the hydrolysis of membrane phospholipids at the *sn*-2 position to release arachidonic acid, which is a rate-limiting step in the production of prostaglandins and leukotrienes [1]. Low-molecular-mass (14 kDa) PLA_2 in mammalian tissues can be classified into two types on the basis of primary structure, group I PLA_2 (PLA_2-I) and group II PLA_2 (PLA_2-II). The latter may participate in the inflammatory process in various disorders such as rheumatoid arthritis [2], pancreatitis [3], Gram-negative septic shock [4], and inflammatory bowel disease [5], and it is believed to be a proinflammatory enzyme [6]. Recently, it was reported that high amounts of PLA_2-II were produced in response to interleukin-1 (IL-1) and tumor necrosis factor, which are potent inflammatory mediators, in rat renal mesangial cells [7] and human synovial fibroblasts [8]. Moreover, these peptide growth factors enhanced not only the activity of PLA_2-II but also the production of mRNA encoding PLA_2-II in rabbit chondrocytes [9] and rat vascular smooth muscle cells [10].

It has been known for many years that periodontitis and rheumatoid arthritis share many common features, for example, bone resorption, and high levels of prostaglandin E_2 [11,12] and IL-1 [13,14] in the diseased regions. In a previous study, we demonstrated PLA_2 activity in the soluble fraction from rat healthy gingival tissue, and suggested that it could be PLA_2-II in terms of substrate specificity, Ca^{2+} dependence, optimum pH and sensitivity to detergent [15], but the details remained unclear. In the present study, we characterized PLA_2-II activity produced and secreted extracellularly by rat gingival fibroblasts in response to human recombinant IL-1β, and furthermore using antisera against PLA_2-I and PLA_2-II, we showed that the activity belongs to the PLA_2-II family.

Abbreviations: PLA_2, phospholipase A_2; IL-1β, interleukin-1β; PE, phosphatidylethanolamine.

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2. MATERIALS AND METHODS

2.1. *Materials*

Recombinant IL-1β was purchased from Genzyme Co., Boston, MA. 1-Acyl-2-[1-^{14}C]arachidonyl phosphatidylethanolamine (PE) was obtained from Amersham International, UK.

2.2. *Cell culture*

Gingival fibroblasts were obtained from male Wistar rats by the method of Wilhelm et al. as described previously [16]. Briefly, the cells were grown in Dulbecco's modified Eagle medium containing 10% fetal calf serum, ascorbic acid (50 μg/ml) and antibiotics. Subconfluent monolayers of cells ($4–6 \times 10^5$ cells/60-mm dish) at passages 3–6 were used.

2.3. *Treatment of cells with IL-1β and assay for PLA_2 activity*

To determine the effect of IL-1β on the levels of PLA_2 activity in both cells and medium, the cells were washed twice with serum-free medium and then cultured in serum-free medium with various concentrations of IL-1β for up to 48 h. At the end of the incubation, the medium was withdrawn and centrifuged at $250 \times g$ for 10 min. The supernatant was removed and stored at −70°C until assay of PLA_2

activity in the medium. On the other hand, for determination of intracellular PLA_2 activity, the cells were washed twice with phosphate-buffered saline and scraped off with a rubber policeman in 50 mM Tris-HCl (pH 7.4). The collected cells were then sonicated (15 s × 2) in ice-cold water, followed by centrifugation at 1,500 × g for 30 min and the supernatant was used as the enzyme source. The assay of PLA_2 activity was carried out by the modified methods of Nishijima et al. [17] and Shaker [18]. Briefly the assay mixtures contained 50 mM Tris-HCl (pH 7.4), 10 mM $CaCl_2$, 5 μM PE and the enzyme. PLA_2 activity was determined by counting the radioactivity of [1-^{14}C]arachidonic acid released.

2.4. *Immunochemical procedures*

Rat antisera against rat pancreatic PLA_2 (anti-PLA_2-I) and rat splenic PLA_2 (anti-PLA_2-II) and its IgG fractions (anti-PLA_2-II IgG) were prepared, and purified as described previously [19]. Each anti-PLA_2 serum (55 mg protein/ml) and IgG (13.3 mg protein/ml) were diluted 10- to 1,000-fold with phosphate-buffered saline. In the PLA_2 inhibition experiments, 40 μl of medium containing PLA_2 activity was incubated with 10 μl of the diluted anti-PLA_2 antiserum or anti-PLA_2-II IgG for 1 h at room temperature. After the incubation, an aliquot was taken to determine the activity of PLA_2. Immunoblot analyses were performed with anti-PLA_2-II IgG as described previously [5].

3. RESULTS AND DISCUSSION

To investigate the effect of IL-1β on the levels of PLA_2 activity in either the cell layers or media, cultures were exposed to various concentrations of IL-1β from 1 to 500 U/ml for 24 h as described in Materials and Methods. Fig. 1 shows the dose–response effects of IL-1β on PLA_2 activity in both the intracellular (cell layers) and extracellular (media) fractions. IL-1β increased PLA_2 activity significantly over each of the control levels in a dose-dependent manner between 5 and 100 U/ml, showing maximal activity at 100 U/ml and over in both fractions. Twelve- and 67-fold increases in PLA_2 activity over the control were observed at 100 U/ml IL-1β in the cell layers and media, respectively. A half-maximal increase (EC_{50}) was estimated to require 20 U/ml IL-1β (5.71 pM). A similar EC_{50} value of IL-1β has been reported to induce PLA_2-II activity in rabbit chondrocytes, with a maximum induction of 13 times the control level after 24 h of incubation with IL-1β [9].

In the time-course experiments shown in Fig. 2, there was a lag period of 6 h after the addition of IL-1β (50 U/ml), and thereafter the PLA_2 activity increased with time between 6 and 24 h. About 5-fold higher activity in the medium than that in the cell layers was observed at any of the times examined during the experimental period for up to 48 h. Either actinomycin D or cycloheximide inhibited dose-dependently the PLA_2 activity

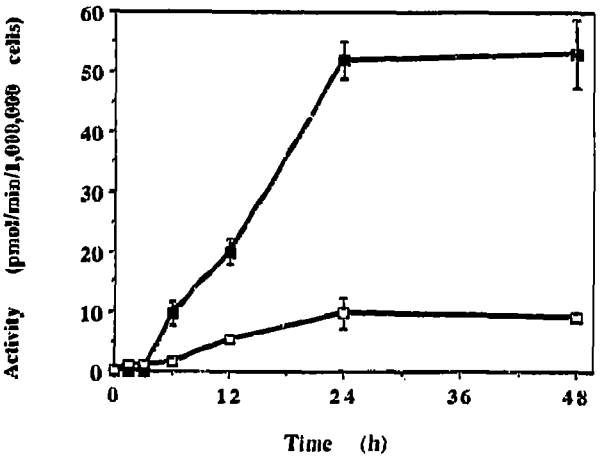

Fig. 2. Time-course effect of IL-1β on intracellular and extracellular PLA_2 activity. Rat gingival fibroblasts were incubated with IL-1β (50 U/ml) for the indicated time periods. PLA_2 activity in the medium (■) and in the cell layers (□) was determined as described in Materials and Methods. Results are means ± S.E.M. for a representative of 3 independent experiments done in triplicate.

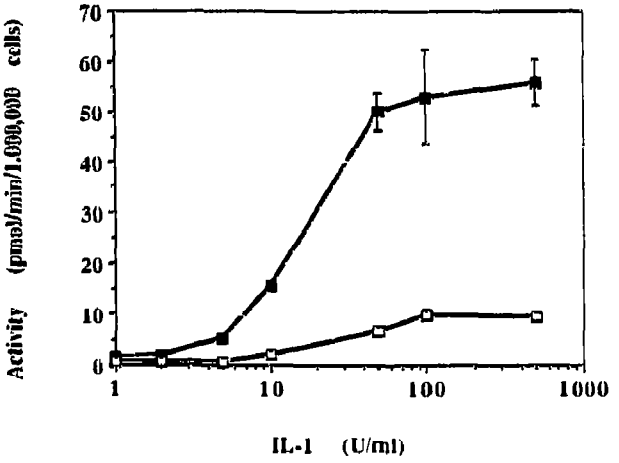

Fig. 1. Dose–response effects of IL-1β on intracellular and extracellular PLA_2 activity. Rat gingival fibroblasts were incubated for 24 h with various concentrations of IL-1β as indicated in the figure. PLA_2 activity in the medium (■) and in the cell layers (□) was determined as described in Materials and Methods. Results are means ± S.E.M. for a representative of three independent experiments done in triplicate.

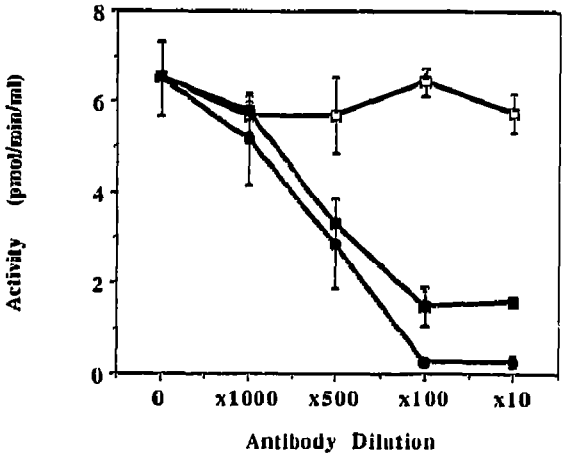

Fig. 3. Effects of the antisera on extracellular PLA_2 activity. Rat gingival fibroblasts were incubated with IL-1β (50 U/ml) for 24 h. Then the medium was incubated with diluted antiserum against PLA_2-I (□) or PLA_2-II (■), or IgG against PLA_2-II (●) for 1 h. After the incubation, the residual PLA_2 activity was assayed as described in Materials and Methods. Results are means ± S.E.M. for a representative of 3 independent experiments done in triplicate.

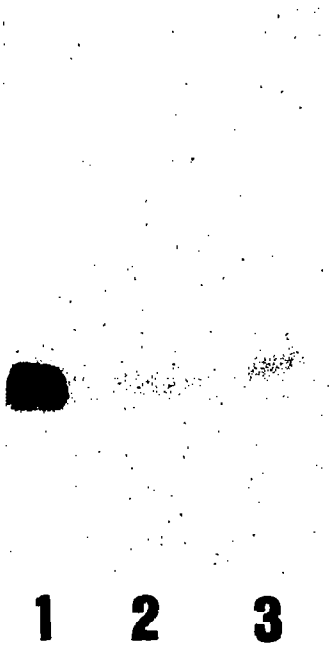

Fig. 4. Immunoblot analysis of PLA_2 secreted from rat gingival fibroblasts after IL-1β treatment. Lane: (1) PLA_2-II purified from rat spleen; (2 and 3) PLA_2 partially purified and enriched from culture media with an S-Sepharose column (5 and 10 μg, respectively). Lanes 2 and 3 were stained for a longer time than lane 1 because of their low content of fibroblast PLA_2.

in the presence of IL-1β (50 U/ml) in the media and the cells (data not shown). Total suppression of IL-1β-stimulated PLA_2 activity to the basal level occurred at 100 ng/ml actinomycin D or 10 μg/ml cycoheximide. The viability of the cells treated with IL-1β was tested by Trypan blue dye exclusion and was found to be more than 96% (data not shown). These findings suggest that the intracellular PLA_2 produced through de novo protein synthesis is rapidly secreted from the cell into the medium after its induction.

The characteristics of rat gingival PLA_2 induced by IL-1β with regard to substrate specificity, optimum pH (pH 8–9) and strict Ca^{2+} requirement (1 mM) are well consistent with those of PLA_2-II in human synovial fluid [20] and pleural effusion [21] as well as those of PLA_2-II in rat spleen [22] and platelet [23] (data not shown). This strongly suggests that the IL-1β-induced PLA_2 in cultured gingival cells could belong to the PLA_2-II family.

To confirm this, we used antisera against rat pancreatic PLA_2-I and rat splenic PLA_2-II. Fig. 3 shows the effects of antisera against PLA_2-I and PLA_2-II on the extracellular PLA_2 activity. The anti-PLA_2-II antiserum inhibited the enzyme activity dose-dependently over a serum dilution range of 1000- to 100-fold, whereas the anti-PLA_2-I antiserum did not. Moreover, the anti-PLA_2-II IgG abolished the enzyme activity induced by IL-1β almost completely (96%). Fig. 4 shows the results of immunoblot analysis using anti-PLA_2-II IgG. PLA_2-II in culture media of gingival cells stimulated by IL-1β was partially purified and enriched with an S-Sepharose column as reported previously [22]. The antibody reacted well with the fibroblast PLA_2, which gave a band showing the same mobility as the PLA_2-II purified from rat spleen. All of these data indicate that the extracellular and intracellular forms of the IL-1β-induced PLA_2 are indistinguishable from each other.

It has been reported that IL-1β also increases PLA_2 activity in other fibroblastic cells, i.e. human foreskin fibroblasts [24], BALB/c3T3 cells [25] and human synovial fibroblasts [26]. However, the magnitude of the increases produced by treatment of these cells with IL-1β was at most 2-fold, far less than that observed in gingival cells (12-fold). It should be noted that no PLA_2 was released into media from human foreskin fibroblasts upon stimulation by IL-1β, but addition of IL-1β to culture medium of human synovial cells resulted in marked stimulation of extracellular PLA_2 activity by 5.8–370-fold [27]. These findings suggest that IL-1β-elicited synthesis and secretion of PLA_2-II from cultured fibroblasts may not be a common feature among fibroblasts, and that the extents of stimulation vary according to the origins of the fibroblasts. Interestingly, evidence for tissue-specific induction of PLA_2-II by lipopolysaccharide in the liver has been reported [28].

In conclusion, the results of this study suggest that IL-1β takes part in eliciting PLA_2-II synthesis possibly through increased protein and mRNA synthesis, and that subsequently the enzyme secreted extracellularly in inflamed sites plays a role in the process of periodontal inflammation.

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REFERENCES

[1] Van den Bosh, H. (1980) Biochim. Biophys. Acta 604, 191–246.
[2] Pruzanski, W., Keystone, E.C., Sternby, B., Bombardier, C., Snow, K.M. and Vadas, P. (1988) J. Rheumatol. 15, 1351–1355.
[3] Nevalainen, T. (1980) Scand. J. Gastroenterol. 15, 641–650.
[4] Vadas, P., Pruzanski, W. and Stefanski, E. (1988) Agents and Actions 24, 320–325.
[5] Minami, T., Tojo, H., Shinomura, Y., Tarui, S. and Okamoto, M. (1992) Gut (in press).
[6] Pruzanski, W. and Vadas, P. (1991) Immunol. Today 12, 143–146.
[7] Pfeilschifter, J., Pignat, W., Vosbeck, K. and Märki, F. (1989) Biochem. Biophys. Res. Commun. 159, 385–394.
[8] Godfrey, R.W., Johnson, W.J. and Hoffstein, S.T. (1987) Biochem. Biophys. Res. Commun. 142, 235–241.
[9] Lyons-Giordano, B., Davis, G.L., Galbraith, W., Pratta, M.A. and Arner, E.C. (1989) Biochem. Biophys. Res. Commun. 164, 488–495.
[10] Nakano, T., Ohara, O., Teraoka, H. and Arita, H. (1990) FEBS Lett. 261, 171–174.
[11] Goodson, J.M., Dewhirst, F.E. and Brunetti, A. (1974) Prostaglandins 6, 81–85.

[12] Trang, L.E., Granström, E. and Lövgren, O. (1977) Scand. J. Rheumatol. 6, 151–154.
[13] Meikle, M.C., Heath, J.K. and Reynolds, J.J. (1986) J. Oral Pathol. 15, 239–250.
[14] Wood, D.D., Ihrie, E.J., Dinarello, C.A. and Cohen, P.L. (1983) Arthritis and Rheumatism 26, 975–983.
[15] Shinohara, H., Ishida, H., Fernandez, E.J., Amabe, Y., Nagata, T. and Wakano, Y. (1992) J. Periodont. Res. (in press).
[16] Wilhelm, S.M., Javed, T. and Miller, R.L. (1983) J. Periodont. Res. 18, 11–22.
[17] Nishijima, J., Okamoto, M., Ogawa, M., Kosaki, G. and Yamano, T. (1983) J. Biochem. 94, 137–147.
[18] Shakir, K.M.M. (1980) Anal. Biochem. 114, 64–70.
[19] Okamoto, M., Ono, T., Tojo, H. and Yamano, T. (1985) Biochem. Biophys. Res. Commun. 128, 788–794.
[20] Hara, S., Kudo, I., Chang, H.W., Matsuta, K., Miyamoto, T. and Inoue, K. (1989) J. Biochem. 105, 395–399.
[21] Baek, S.H., Takayama, K., Kudo, I., Inoue, K., Lee, H.Y., Do, J.Y. and Chang, H.W. (1991) Life Sci. 49, 1095–1102.
[22] Ono, T., Tojo, H., Kuramitsu, S., Kagamiyama, H. and Okamoto, M. (1988) J. Biol. Chem. 263, 5732–5738.
[23] Horigome, K., Hayakawa, M., Inoue, K. and Nojima, S. (1987) J. Biochem. 101, 53–61.
[24] Ballou, L.R., Barker, S.C., Postlethwaite, A.E. and Kang, A.H. (1990) J. Immunol. 145, 4245–4251.
[25] Burch, R.M., Connor, J.R. and Axelrod, J. (1988) Proc. Natl. Acad. Sci. USA 85, 6306–6309.
[26] Godfrey, R.W., Johnson, W.J., Newman, T. and Hoffstein, S.T. (1988) Prostaglandins 35, 107–114.
[27] Gilman, S.C., Chang, J., Zeigler, P.R., Uhl, J. and Mochan, E. (1988) Arthritis and Rheumatism 31, 126–130.
[28] Inada, M., Tojo, H., Kawata, S., Tarui, S. and Okamoto, M. (1991) Biochem. Biophys. Res. Commun. 174, 1077–1083.